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Research Article

First recordings of the ctenophore *Euplokamis* sp. (Ctenophora, Cydippida) in Swedish coastal waters and molecular identification of this genus

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Abstract

The ctenophore *Euplokamis* sp. was observed and collected in the Gullmar fjord on the west coast of Sweden in April-August 2011 during regular jellyfish and zooplankton monitoring. In April-May 2011, both larval and adult *Euplokamis* sp. were collected in the surface water of the fjord and in June-August in the deep part of the fjord at 110-100 m. The typical morphological and behavioral characters of *Euplokamis* sp. were observed (i.e. coiled tentacle side branches and rapid swimming both in forward and reverse). With no published sequence data from the ctenophore family Euplokamidae, molecular identification using the nuclear 18S rRNA gene and internal transcribed spacer region I region (ITS1) revealed that *Euplokamis* sp. individuals were closely related to the morphologically similar ctenophore family Mertensiidae. Therefore, morphological and molecular data support the identification as *Euplokamis*. *Euplokamis* sp. densities in the deep water of the Gullmar fjord in June 2011 reached similar densities as the local ctenophore *Beroe* spp. The presence of *Euplokamis* sp. and other ctenophores in the area is discussed together with the importance of continuous monitoring of gelatinous forms to include detection of less frequent and more fragile ctenophore species. We also discuss the importance of molecular identification for ctenophore larvae and adult ctenophores of small size.

Key words: *Euplokamis*; Ctenophora; monitoring of jellyfish; Gullmar fjord; Swedish west coast

Introduction

The ctenophore genus *Euplokamis* was first described by Chun 1879 and belongs to the family Euplokamidae (Mills 1987). Within the genus the most described species is *Euplokamis dunlapae* (Mills 1987) which is a small (max 2 cm oral-aboral length) cydippid ctenophore occasionally found in surface waters of fjords and inlets on the west coast of the US (Washington State) and Canada (British Columbia) (Mackie et al. 1988; Mills 1987). *E. dunlapae* is commonly observed at depths below 100 m in the northeast Pacific, reaching its highest abundances below 250 m (Mackie et al. 1988; Mills 1987). Throughout the Strait of Georgia and in inlets on the coast of British Columbia, *E. dunlapae* is one of the most common midwater ctenophores, frequently observed from submersibles, in densities up to 10 ind m^{-3} (Mackie and Mills 1983; Mackie 1985). *E. dunlapae* has been recorded also in the NW Atlantic (Gulf of Maine) in the 1990´s (C Mills, pers. comm.).

In Europe another species from the Euplokamidae family, *Euplokamis stationis* (Chun, 1879) is described from the Mediterranean Sea: the Tyrrhenian Sea (Chun 1879; Madin 1991) and the Alborán Sea (Mills et al. 1996). *E. stationis* in the Mediterranean Sea is described from depths ranging from about 200-800 m and considered a true deep-sea species that can be brought to the surface by upwelling (Mills et al. 1996). According to Mills et al. 1996 this species may be common although only rarely reported.

Also, *Euplokamis* sp. has been recorded with submersibles and caught by net in fjords on the west coast of Norway in recent years (P. R.

Flood and U. Båmstedt, pers. comm.). Here we do not aim to determine the *Euplokamis* to species level but just to report the first findings of the genus *Euplokamis* in Swedish waters, and for the first time, report the 18S rRNA gene and ITS1 region of a Euplokamidae species.

The hitherto-recorded ctenophores in the Gullmar fjord at the Swedish west cost are *Pleurobrachia pileus* (O. F. Müller, 1776), *Bolinopsis infundibulum* (O.F. Müller, 1776), *Beroe cucumis* (Fabricius, 1780) and *Beroe gracilis* (Künne, 1939) and since 2006 the introduced *Mnemiopsis leidyi* (Agassiz, 1865). Ctenophore species, especially small specimens i.e. cydippid stage larvae, are difficult to identify using solely morphological methods (Gorokhova and Lehtiniemi 2010). The recently misidentified *Mertensia ovum* (Fabricius, 1780) has been reported to occur in the northern Baltic Sea/Swedish east coast (Gorokhova et al. 2009) which are adjacent waters to the present study area. *M. ovum* and *P. pileus* are both cydippid ctenophores which morphologically are similar to *Euplokamis* sp.

Methods

Sampling

Sampling was conducted in the Gullmar fjord on the west coast of Sweden (58°15´N; 11º27´E) (Figure 1 A and B). The Gullmar fjord is a relatively shallow fjord (120 m deep, entrance sill 45 m) and the water column is permanently stratified with a surface layer (0 to 20 m) of variable salinity (15 to 27 psu), an intermediate layer (20 to 50 m; 27 to 32 psu) and a stagnant deep layer (50 to 120 m; 32 to 34 psu). The conditions in the upper 50 m of the fjord resemble the surrounding coastal waters.

During 13-14 April, 20-21 June and 10-11 August 2011 stations Släggö (70 m) and Alsbäck (120 m) was sampled with R/V Skagerak (Figure 1 B). At station Släggö sampling of ctenophores was conducted with duplicate vertical tows (20-0 m) using a 450 µm WP-3 net with a filtering cod-end (with the mesh on the side) as a part of a regular jellyfish/zooplankton monitoring program (Møller and Tiselius unpublished data). At Alsbäck a Hydrobios multinet was used, with a similar cod end as described above. The nets were towed vertical (90 µm) or oblique (300 µm) at 5 different depths (112-100m, 100-80m, 80-50m, 50-20m, 20m - surface)

During three occasions (20 April, 4 and 9 May 2011) *Euplokamis* sp. were collected with beakers from the surface water at the jetty outside Sven Lovén Centre for Marine Sciences – Kristineberg. The ctenophores were brought to the laboratory, measured and kept together with wild zooplankton (mainly *Calanus* sp.) at 6ºC.

Molecular species identification

To identify the species, we used 6 randomly selected individuals collected in May 2011. DNA was extracted from tissue preserved in 70-80% ethanol with a modified Chelex rapid-boiling procedure (Walsh et al. 1991; Jarman et al. 2002). Approximately 0.5 mg of tissue was placed in a 1.5-mL micro centrifuge tube containing 30 µL of 5% Chelex® 100 resin (Bio-Rad) in 50 mM Tris, pH 8.0, and 0.5 mM EDTA. The tube was heated at 98°C for 10 min and then centrifuged at 20000×g for 10 min. The resulting supernatant was removed to a new 1.5-mL centrifuge tube. The ITS1 (<300 basepairs, bp) and 18S (app. 1800 bp) PCR amplifications were performed three separate times per individual on an MJ Research PTC 100 Thermal Cycler using primers for *Mnemiopsis leidyi*, *Pleurobrachia pileus* and *Mertensia ovum* ITS region (Gorokhova et al. 2009), universal eukaryotic primers for ITS1 (Podar et al. 2001) and 18S (Kober and Nichols 2007). PCR of 20 µL contained 0,4 µL Phire® Hot Start DNA polymerase, 4 µL of Phire ® reaction buffer, 0,4 µL of each primer (final concentration 0.2 mmol), 1 µL of DNA template, 0,4 µL of DNTP, 0,6 µL of 3% DMSO and 12,8 µL nuclease-free water. The cycling regime was as follows: initial denaturing period of 5 min at 98°C followed by 32 cycles of 98°C (5s), 64°C (5s), 72°C (20s), with a final extension for 2 min at 72°C. PCR products were purified using the Montage PCR_{96} Cleanup Kit (Millipore) according to the manufacturer's instructions. Cycle sequencing of the PCR products was carried out in Macrogen Sequencing Service (Macrogen Inc., South Korea) with the primers mentioned above. Due to the long length of 18S sequence, an additional sequencing primer was designed to the middle of the sequence (18SM 5'-GTG TAC TGA TCG ATC TGT TC-3'). Although, each section of the sequences was not sequenced in both directions, each individual was sequenced from three separate PCRs. The resulting nucleotide sequences were assembled using BioEdit software (Hall 1999) and electropherograms were checked by eye for poor base calls and sequence quality.

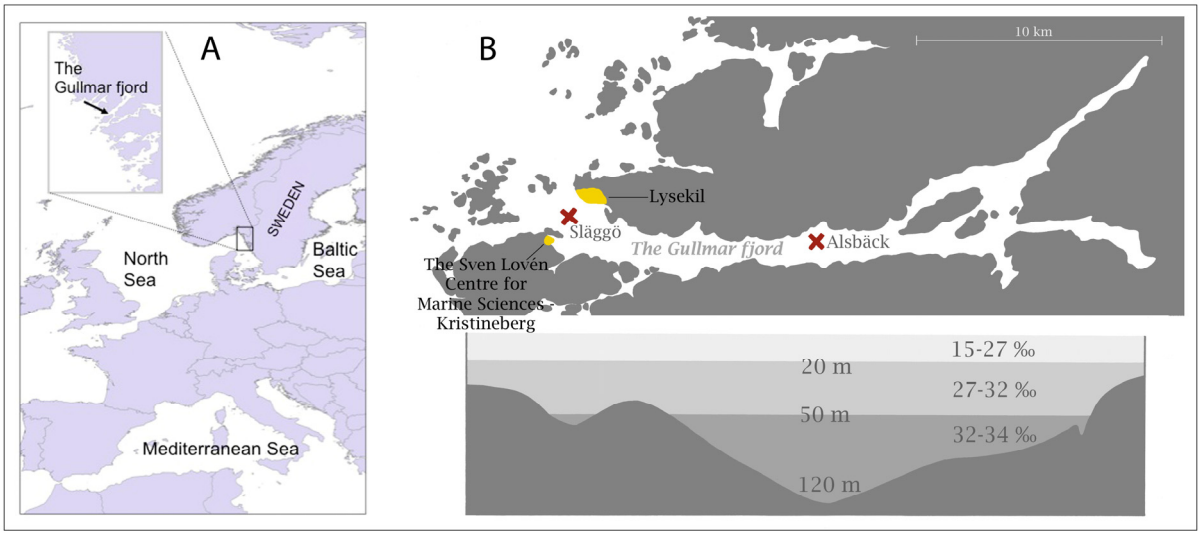

Figure 1. Map showing the location of the Gullmar fjord (**A**) at the Swedish west coast and the sampling staions (**B**).

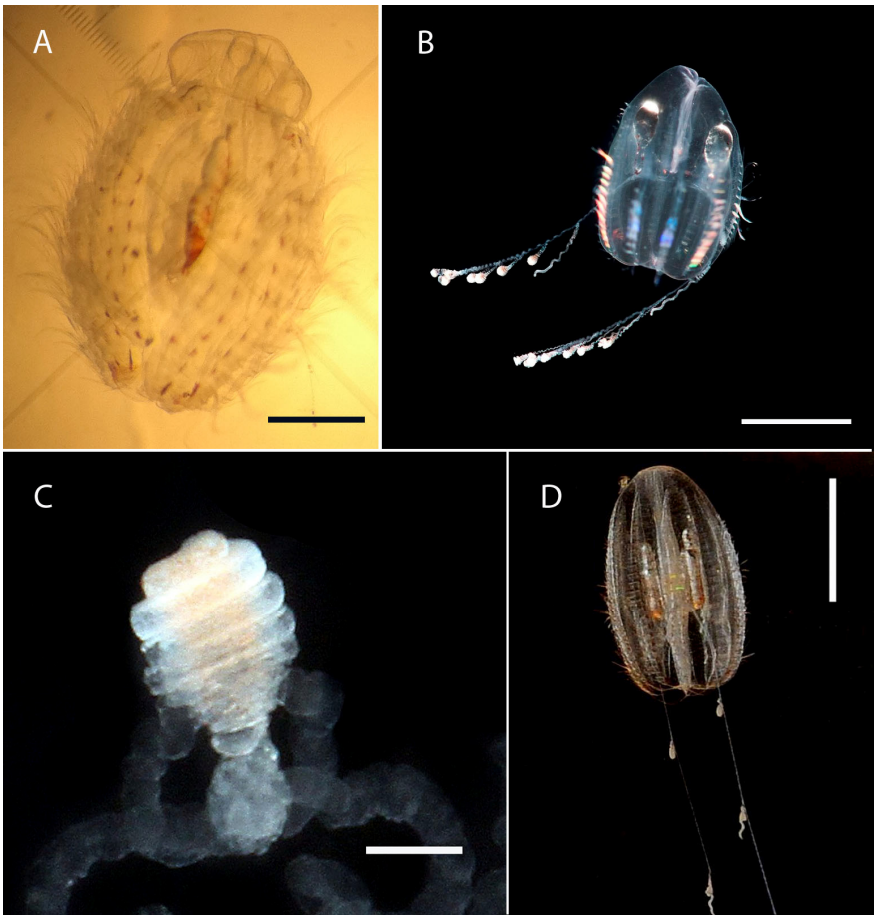

Figure 2. *Euplokamis* sp. individual at different developmental stages: **A**) 3 mm larvae (scale bar 1 mm). Note the large tentacle bulb in the centre; **B**) 8 mm adult (scale bar 5 mm). Note the coiled tentilla on the two tentacles; **C**) detail of tentillum on the tentacle, length 0.5 mm (scale bar 0.1 mm); **D**) 10 mm adult (scale bar 5 mm). Photographs by L. Granhag (**A**), Erik Selander (**B**, **C**) and Susan Gotensparre (**D**).

The sequences were aligned using MAFFT (Katoh et al. 2002) with Q-INS-i strategy, which takes RNA secondary structure into account, and gap-opening penalty of 1.53 and gap extension penalty of 0.123. Since 18S gene is extremely conservative within ctenophores (Podar et al. 2001), the alignment includes only a few variable sites. Maximum likelihood bootstrap support values were calculated from 1000 replicates for 18S rRNA gene (alignment 1723 bp) and ITS1 region (452 bp) using GARLI 1.0.659 (Zwickl 2006) with model Tim2+I+G and TIM3ef+G, respectively. The models were selected in jModelTest 0.1.1 (Posada 2008) with AICc criterion. Posterior probabilities for 18S rRNA gene were calculated with MrBayes 3.2 (Ronquist and Huelsenbeck 2003) from two independent runs with four Markov chains and 10000000 generations with the first 25% of the sampled trees discarded, leaving 150002 trees. Since MrBayes cannot implement the Tim2+I+G model, a similar model (GTR+I+G) was used. The sequences reported in this paper have been deposited in the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (GenBank Accession number HE647719 *Euplokamis* spp. genomic DNA containing 18S rRNA gene and HE805698 and HE805699 containing ITS1).

Results and discussion

Recordings in the Gullmar Fjord

During seven occasions in April to August 2011 *Euplokamis* sp., all in good condition, were observed within an ongoing monitoring program of jellyfish and ctenophores in the Gullmar fjord (Table 1). The first records of *Euplokamis* sp. were made in surface waters at the station Släggö in the outer part of the fjord on 13 and 14 April. During the same sampling period no individuals were found at Alsbäck station (Figure1A and B). *Euplokamis* sp. was also observed on 18 April, in Lysekil harbour on the northern side of the Gullmar fjord, likewise at the entrance of the fjord (F. Norén, pers comm). In April and May adult *Euplokamis* sp. were collected with beakers from the surface water at the jetty outside Sven Lovén Centre for Marine Sciences – Kristineberg. In June *Euplokamis* sp. were collected in densities reaching 4.9 ind m^{-3} at the deep part of at the Alsbäck station (primarily at depth 112-100 m, Table 2) while no individuals were observed at the Släggö station. The size distribution varied from 3-11 mm (Figure 3). In August only a single specimen of *Euplokamis* sp. were collected from the deep part (110-100 m) of Alsbäck and no other ctenophores were observed and again none at Släggö. Within the period when *Euplokamis* sp. were collected, temperature varied between 5-13°C and salinity between 19-34 psu (Table 1). The abundance of *Euplokamis* sp. almost reached the same abundance as the native *Beroe* sp. collected at the same time, location and depth (Table 2).

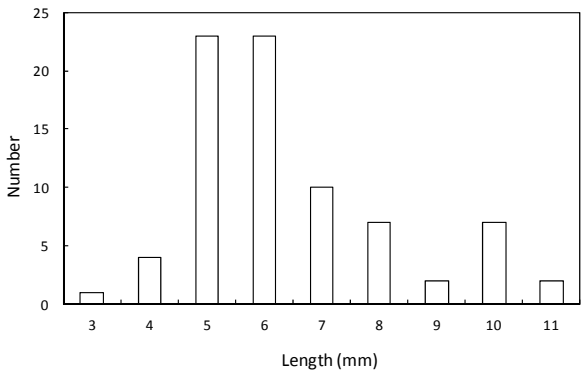

Figure 3. *Euplokamis* sp. size distribution (L, mm) of 79 individuals collected at 110-100 m at Alsbäck, Gullmar fjord, 21 June 2011.

Morphological identification

The first specimens collected from the surface waters on 13 and 14 April were 2-3 mm in oral-aboral length (Figure 2A). The *Euplokamis* larvae had large tightly-packed cilia giving a "furry" look which differs from other ctenophore larvae like *Mnemiopsis leidyi*/*Bolinopsis infundibulum* (Lobata) and *Beroe* spp. (Beroida) or larvae or small sizes of adult ctenophores i.e. *Pleurobrachia pileus*/*Mertenisa ovum* (Cydippida) present in this and adjacent waters. The *Euplokamis* larvae also had red pigments along the comb rows and tentacle bulbs containing oil and red pigments. Embryos and larvae of *Euplokamis dunlapae* are described in Mills (1987), where 1.5 mm long cydippid larvae had assumed most of the adult characteristics like rapid tentacle withdrawal and swimming. The specimens collected in the Gullmar fjord had developed the characteristics of the adults with tentacle bulbs and tentacles exiting the body towards the aboral end but the furry look due to

Table 1. *Euplokamis* sp. collections and observations in the Gullmar fjord, on the west coast of Sweden, April-August 2011. Number and length (oral-aboral) of ctenophores, collection method used, depth (m), temperature (°C) and salinity at site of collection.

Date	Number of *Euplokamis* sp. collected	Length, oral-aboral (mm)	Collection method	Depth (m)	Temperature (°C)	Salinity psu
13 April	3	3-4	plankton net	20-0	5	30
14 April	4	3-4	plankton net	20-0	5	30
20 April	4	8-12	beaker	surface	9	21
4 May	1	12	beaker	surface	10	20
9 May	2	10-12	beaker	surface	13	19
20 June	79	3-11	multinet	110-100	6	34
10 August	1	ca 10	multinet	110-100	6	34

Table 2. Abundance of *Euplokamis* sp. and *Beroe* sp. collected with Multinet (Hydrobios) at different depths at Alsbäck, the Gullmar fjord, 21 June 2011. #1 och #2 vertical tows 90 µm net , #3 oblique tow 300 µm net.

Depth (m)	*Euplokamis* sp. (ind m^{-3})			*Beroe* sp. (ind m^{-3})		
	#1	#2	#3	#1	#2	#3
20-0	0	0	0	0	0	0
50-20	0	0	0	0	0	0.1
80-50	0	0	0	1.0	0	1.2
100-80	0	0.2	0.2	5.0	5.2	4.3
112-100	4.9	2.4	2.0	1.8	1.3	0.7

the large and tightly packed cilia gave them a juvenile impression (Figure 2A). The collected 3 mm individuals grew to a size of 12 mm ctenophores within approximately 7 days when maintained with copepods (primarily *Calanus* sp.) at 6°C in the fridge. They survived one month in a beaker with seawater, where copepods and water were renewed few times. The adults, both the ones grown from larvae in the lab and the field collected adults were 8-12 mm in oral-aboral length. *Euplokamis dunlapae* of size up to 20 mm is described (Mills and Haddock 2007) and *Euplokamis stationis* of size up to 25 mm (Chun 1879).

The adults had unpigmented or lightly pink tentacles and a transparent body. The specimens had, as is typical of cydippid ctenophores, a pair of tentacles exiting the body towards the aboral end (opposite the mouth) (Figure 2B). The tentacles had fewer side branches (tentillae) compared to e.g. *Mnemiopsis* cydippid larva or *Pleurobrachia pileus* and side branches were coiled up (Figure 2C) except when it was capturing prey. The ctene rows of the collected specimens constituted approximately ¾ of the total length and larger specimens were more elongated (larger length to width ratio) than the smaller specimens. The swimming of *Euplokamis* sp. was seen to be much more rapid than swimming in other cydippid ctenophores (i.e *Pleurobrachia pileus* and *Mertensia ovum*). The rapid swimming and the food capture mechanism of the coiled tentilla in *Euplokamis dunlapae* are described by Mackie et al. (1992, 1988).

The cydippid ctenophores *Mertensia ovum* and *Pleurobrachia pileus* of the same size as the observed *Euplokamis* sp. (12 mm) differ morphologically from *Euplokamis* sp. both by having uncoiled tentillae and a different body shape (more flattened for *Mertensia* and more spherical for *Pleurobrachia*). While *Pleurobrachia pileus* during periods is common in the Gullmar fjord, no *Mertensia ovum* of this size has been recorded during the ctenophore monitoring.

The most characteristic morphological feature of *Euplokamis* sp. is the coiled tentilla on the tentacles (Figure 2C), giving the tentacle a beaded appearance when viewed from a distance. The tentilla are used for prey capture, they are held tightly coiled when relaxed but stretch out at high velocity when triggered by contact with prey (Mackie et al. 1992). The tentillum of *Euplokamis dunlapae* is considered a true food-capturing organ and it is probably the most highly developed organ among the ctenophores

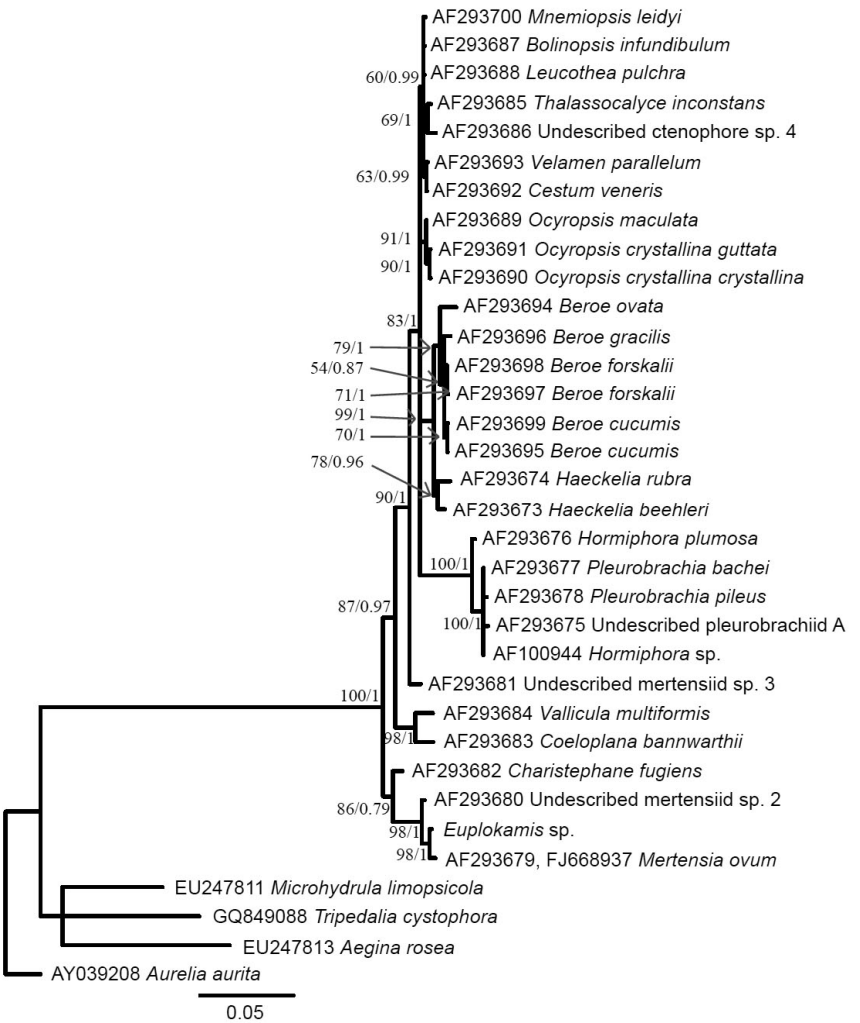

Figure 4. Maximum-likelihood tree for 18S (1723bp) from all sequenced ctenophore species in GenBank including the maximum likelihood bootstrap (TIM2+I+G in Garli) and Bayesian posterior probability values (GTR+I+G in MrBayes). The tree was rooted with *Aurelia aurita, Aegina rosea*, *Tripedalia cystophora* and *Microhydrula limopsicola* as the outgroup. Horizontal branch lengths reflect genetic distances among taxa.

(Mackie et al. 1988). The tentillum extension in *E. dunlapae* is very rapid, and controlled by striated and smooth muscle cells (Mackie et al. 1988). This comb jelly moves fast compared to other cydippid ctenophores, both forward and in reverse. The rapid movement is generated by accelerated cilia beating when moving forward or by changed direction of cilia beating when moving backwards (Mackie et al. 1992). The cilia beating is controlled by giant neurons that run along each comb row. By the linking of giant neurons with smaller neurites of the nerve plexus, with each other and with the ciliated cells of the comb plates, they appear to form a single system mediating signal transfer in both directions (Mackie et al. 1992).

Molecular identification

As the morphological identification of larvae or small adult specimens of cydippid ctenophore are difficult molecular identification was conducted. In molecular analysis, none of the individuals produced positive ITS1 region amplifications with *M. leidyi* or *P. pileus*-specific primers, therefore both *M. leidyi* and *P. pileus* were ruled out as possible matches. However all individuals were successfully amplified using ITS1 region primers M1F, M1R and M2F, M2R (Gorokhova et al. 2009). To confirm the product identity, samples were subjected to PCR with universal eukaryotic primers for ITS1 region and 18S rRNA gene. All

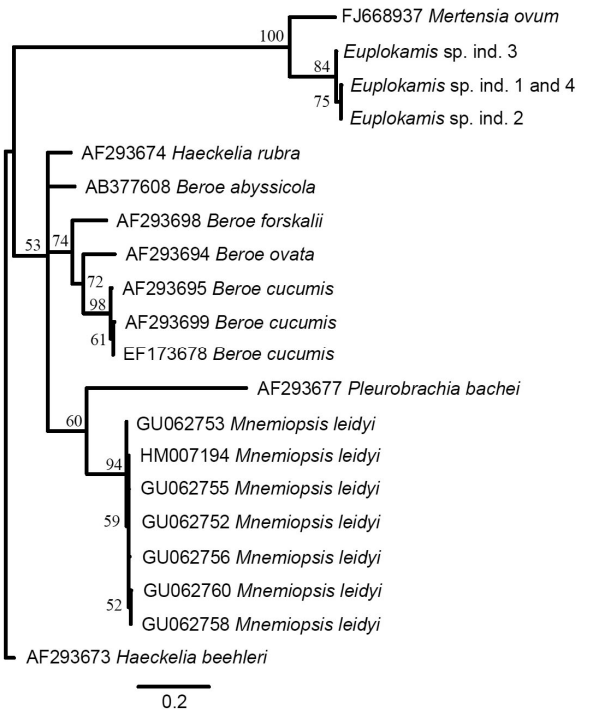

Figure 5. Maximum-likelihood tree for ITS1 region (alignment used 452 bp) from selected ctenophore species in GenBank (see accession numbers) including the maximum likelihood bootstrap values (TIM3ef+G in Garli). Horizontal branch lengths reflect genetic distances among taxa.

six individuals were successfully amplified three times for 18S and four individuals for ITS1. Sequences were obtained for 1863-bp-long region covering the 18S rDNA and partial ITS1 and 614-840-bp-long region covering the ITS1 region and partial 5.8S rDNA. The entire 18S sequence was invariant in all specimens examined, whereas there was some variability between individuals in ITS1 rDNA (max 7 bp). However, the variation was in the range found between *M. ovum* individuals in the Baltic Sea (Gorokhova et al. 2009) and thus, we concluded that all samples contained only one species.

The BLASTN search in the NCBI GenBank database revealed 99% match for the 18S rRNA with *M. ovum* (FJ668937; AF293679) (8 bp difference excluding two ambiguous positions) and undescribed mertensiid sp. 2 (AF293680) (14 bp difference excluding two ambiguous positions), and our sequence clustered together with these mertensiids in the maximum likelihood tree (Figure 4). The 18S rDNA is known to be very conserved among ctenophore species, with lengths between 1801 and 1809 bp, and with a maximum divergence between two species of 87 bp, i.e. less than 5%. Close sequence similarity (only 2 bp differences at the level of the 18S rDNA) has earlier been found between mertensiid *M. ovum*, an Arctic species, and a yet undescribed mertensiid species (species 2) which inhabits the tropics, even though these two species are anatomically quite distinct (Podar et al. 2001). For the ITS1 region, the BLASTN search revealed lower sequence identity (91 %) with *M. ovum* (FJ668937) (51-52 bp difference). Nevertheless, the ITS1 sequences clustered together with *M. ovum* in the maximum likelihood tree (100% maximum likelihood bootstrap support) (Figure 5). Therefore, together with morphological identification, all *Euplokamis* sp. specimens sequenced could be separated from *M. ovum* and undescribed mertensiid sp. 2 specimens sampled in the North Atlantic, in the high Arctic and in the Baltic Sea.

The very short evolutionary distances between characterized ctenophore 18S rRNA gene sequences has allowed us to unambiguously align the consensus 18S sequence with all published sequences for the whole Ctenophora phylum, with each other over the entire length (Podar et al. 2001). All the species commonly found at the Swedish west coast and in the northern Baltic Sea were included to the alignment. However, none of the species in the

family Euplokamidae has been sequenced before. Several subgroups, similarly to Podar et al. 2001, consisting of traditional ctenophore taxa could be distinguished (Figure 4). *Euplokamis* sp. displayed a strong relationship (98% maximum likelihood bootstrap support and 1.0 Bayesian posterior probability) with the family Mertensiidae (*M. ovum* and undescribed mertensiid sp. 2). However, undescribed mertensiid sp. 3 appears to be excluded from this group. Since *M. ovum* is the only described species in the family Mertensiidae, and the variation within the family is unknown based on existing 18S rDNA sequences, it cannot from this analysis be determined if *Euplokamis* sp. belongs to family of Mertensiidae or in separate family (family Euplokamidae) as has been morphologically described (Mills 1987).

On the presence of Euplokamis sp., the importance of monitoring and use of morphological and molecular identification

The exchange process of water in the Gullmar fjord above sill depth is more or less continuous while the deep water in the fjord (below sill level) is renewed completely or partly, with deep water from Skagerrak, North Sea or the Atlantic, in late winter or early spring (Lindahl 1987). It seems as if *Euplokamis* sp. arrived with high saline surface waters in April and then went to the deep part of the fjord.

One year before the observations in the Gullmar fjord, the 13 April 2010, a ctenophore larvae (4 mm), with long ctenes on the ctene rows and "furry" as described earlier, was found during a zooplankton monitoring cruise in Kattegatt (Swedish west coast) close to the island Anholt (56°40´N, 12º 07´E) depth 10-20 m (M. Haraldsson, pers. comm.). From photos of this specimen we identify it as a *Euplokamis* larvae.

As *Euplokamis dunlapae* was recorded by submersibles along the US west coast but not found in plankton tow samples from the same time and area, Mills (1987) concluded that *E. dunlapae* did not preserve well and therefore were not detected in her fixed samples. As most jellyfish are fragile and easily destroyed by both towing in plankton nets and during preservation, they are challenging to survey. As *Euplokamis* sp. probably is native to the NW Atlantic and indigenous in North Atlantic waters off Norway, the more frequent monitoring of jellyfish in the Gullmar fjord in recent years might be the reason for the detection presented here. As shown within this note, monitoring can also reveal new recordings at the larval stage.

We do not know the consequences of the *Euplokamis* sp. observations in the waters at the Swedish west coast or if it is a new arrival or not. The observations of this species can be a result of an ongoing and frequent monitoring program for zooplankton and gelatinous forms and it is likely that is has been sporadically occurring in those areas, in the NA pelagic zone and the periphery of the Baltic, before our observations. Even though *Euplokamis* sp. is capable of reaching high abundances in its native habitat, it might not happen in the Gullmar fjord. To observe which species are present in different areas and to predict consequences of possible new arrivals of species continuous monitoring and live samples of gelatinous forms are needed. With distribution and abundance data the zooplankton-predatory impact exerted by jellyfish/ctenophores can then be investigated.

For identification of ctenophore larvae and small sized adults, the use of molecular methods in addition to morphological identification is needed. As a species in the family Euplokamidae has now been sequenced, molecularly identified and further aligned with all published sequences for the Ctenophora, this can be used in future work in order to reveal relationships within this phylum.

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